J. Pharm. Pharmacol. 1993, 45: 753–755
Communicated November 15, 1992

Selective inhibition of NS-398 on prostanoid production in inflamed tissue in rat carrageenan-air-pouch inflammation

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Abstract—NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl) methane sulphonamide), a newly synthesized potent non-steroidal anti-inflammatory drug (NSAID) has a much lesser degree of toxicity, as compared with presently available NSAIDs. We have investigated the inhibition of prostanoid production in inflammatory exudate, gastric mucosa and renal papillary tissue, following oral administration to carrageenan-air-pouch rats. The ID50 values of NS-398 in the inflammatory exudate, gastric mucosa and renal papillary tissue were 0·18, 62·2 and 261·7 mg kg^{-1}, respectively. In contrast, indomethacin decreased the PGE_2 concentration in the inflammatory exudate, gastric mucosa and renal papillary tissue, with the same dose range, the ID50 values being 0·23, 0·14 and 0·15 mg kg^{-1}, respectively. The same tendency was seen for 6-keto-prostaglandin F_1 and thromboxane B_2. Moreover, NS-398 inhibited excess PGE_2 production in inflamed tissue but did not affect physiological production of PGE_2 in non-inflamed tissue. Indomethacin, in both inflamed and non-inflamed tissues, inhibited PGE_2 production to the same degree. These results indicated that NS-398 has some specificity for inflamed tissue, by inhibiting prostanoid synthesis, and this effect may explain the decreased side-effects of this drug.

NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl) methanesulphonamide) is a newly synthesized non-steroidal anti-inflammatory drug (NSAID) (Futaki et al 1993). While the anti-inflammatory, analgesic and antipyretic effects in several experimental models were almost equipotent with those of indomethacin, diclofenac and loxoprofen, all potent NSAIDs, NS-398 produced much weaker gastro-intestinal and renal adverse damage.

NSAIDs exhibit therapeutic effects mainly by inhibiting prostaglandin biosynthesis, which in turn is related to ulcerogenicity and renal side-effects. Cyclo-oxygenase enzyme preparations from different tissues show differential sensitivities to some compounds in-vitro (Flower & Vane 1974). Whittle et al (1980) reported that some NSAIDs can selectively inhibit prostaglandin biosynthesis in different tissues, in-vivo. The inhibitory effect on prostaglandin production in inflammatory exudate was reported to be more potent than that in gastric mucosa (Imayoshi et al 1984; Tofanetti et al 1989).

In this paper, we compared the effect of NS-398, on production of prostaglandins, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 (thromboxane B_2) in inflammatory exudate, gastric mucosa and renal tissue in carrageenan-air-pouch inflammation in rats.

Materials and methods

Effects of NS-398 and indomethacin on prostanoid content in inflammatory exudate, gastric mucosa and renal tissue in carrageenan-air-pouch inflammation. Carrageenan-air-pouch inflammation was prepared according to the method of Fukuhara & Tsurufuji (1969), with some modification. Briefly, male Wistar rats, 100–150 g, were subcutaneously injected with 8 mL air into the dorsum and 24 h later, 5 mL 1% carrageenan was injected into the air sac to induce inflammation. Drugs were administered orally immediately before the carrageenan injection. Three hours later, the rats were killed, the exudate in the pouch was collected and the volume was measured. Simultaneously, the stomach and the kidney were rapidly removed under conditions of pre-chilling, after which concentrations of prostanoids were measured.

Cell counts in the inflammatory exudates were performed using a haemocytometer and differentials performed on cell preparations stained with Wright.

Prostanoid assays. Exudate (0·5 mL) was mixed with buffer (0·5 mL, pH 6·8) and centrifuged at 3000 rev min^{-1} for 10 min. The supernatants were used for quantitative determinations. The stomach was opened along the greater curvature, washed in saline, pinned out and about 50 mg of mucosal tissue was removed. The kidney was opened and about 10 mg of papillary tissue was removed. These tissues were homogenized in 1 mL ethanol containing 100 μM indomethacin, to inhibit prostaglandin formation during extraction, then centrifuged at 10000 rev min^{-1} for 15 min; the supernatants were used for determinations. All procedures were performed under conditions of pre-chilling. The amounts of PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 (the latter are stable metabolites of PGI_2 and TXA_2, respectively) in these samples were determined by radioimmunoassay using commercial kits (NEN, Boston, MA, USA), based on the use of iodinated analogues as tracer and rabbit anti-prostaglandin as the anti-serum. The radioactivity was measured in a gamma-counter (ARC-300, Aloka, Tokyo, Japan).

Effects of NS-398 and indomethacin on PGE_2 production in inflamed and non-inflamed tissues in rats. Carrageenan-air-pouch inflammation was prepared as above and drugs were administered orally immediately before the carrageenan injection. Three hours later, the animals were killed and the dorsum muscles in the carrageenan-pouch were excised as inflamed tissue. These tissues were washed by Hanks' balanced salt solution (HBSS, LTI, NY, USA) and homogenized in 1 mL HBSS for 15 s to produce PGE_2 and then centrifuged at 10000 rev min^{-1} for 15 min. All these procedures were performed under conditions of pre-chilling. The amount of PGE_2 in the supernatant was measured by radioimmunoassay. The same part of dorsum tissues from non-carrageenan rats was excised as non-inflamed tissue, and the same procedure was carried out. Drugs were administered orally 3 h before the assay.

Drugs. NS-398 synthesized in Taisho Pharmaceutical Laboratories and indomethacin (Sigma, St Louis, MO, USA) were suspended in 5% gum arabic-aqueous solution. Carrageenan (Picnin A; Zushi Kagaku, Kanagawa, Japan) was suspended in saline.

Statistical analysis. ID50 values were defined as doses inhibiting the control value by 50% and were calculated by the least-squares method.

Results

The effects induced by NS-398 and indomethacin on prostanoid levels in inflammatory exudate, gastric mucosa and renal papillary tissue are shown in Fig. 1. The amount of PGE_2 in inflammatory exudate, gastric mucosa and renal papillary tissue,

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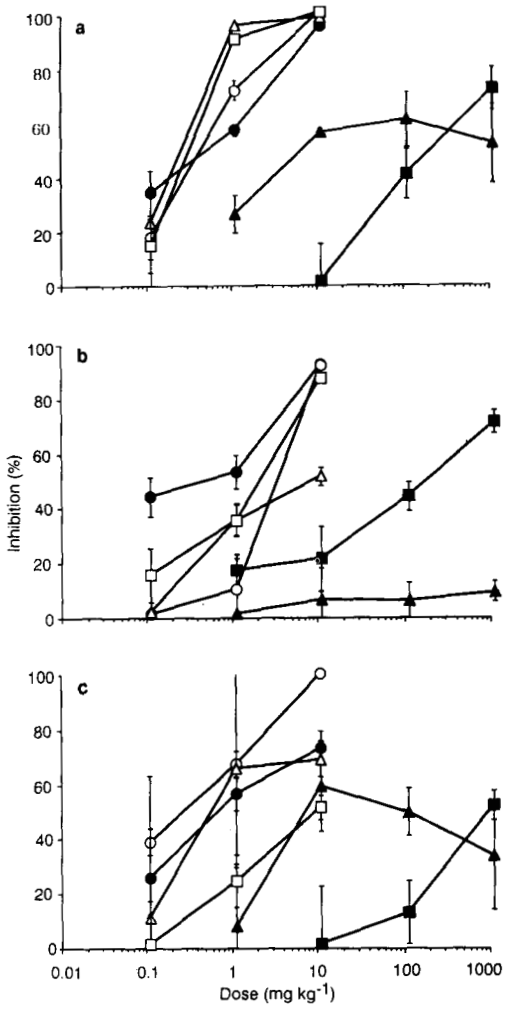

FIG. 1. a. Effects of NS-398 and indomethacin on PGE_2. b. 6-keto-$PGF_{1\alpha}$. c. TXB_2 contents in inflammatory exudate, gastric mucosa and renal papillary tissue in rat carrageenan-air-pouch inflammation. ●, ▲, ■ Inflammatory exudate, gastric mucosa and renal papillary tissue for NS-398, respectively. ○, △, □ Inflammatory exudate, gastric mucosa and renal papillary tissue for indomethacin, respectively.

3 h after the carrageenan injection was $23{\cdot}7 \pm 1{\cdot}6$ ng/rat, $98{\cdot}6 \pm 21{\cdot}5$ ng (g tissue)$^{-1}$ and $6{\cdot}63 \pm 0{\cdot}49$ μg (g tissue)$^{-1}$, respectively. NS-398, 0·1–10 mg kg^{-1}, markedly decreased the content of PGE_2 in the inflammatory exudate, in a dose-dependent manner.

The decrease of PGE_2 content in gastric mucosa and renal papillary tissue was much less than that in the inflammatory exudate and was not completely inhibited even with a dose of 1000 mg kg^{-1}. The ID50 values of NS-398 in the inflammatory exudate, gastric mucosa and renal papillary tissue were 0·18, 62·2 and 261·7 mg kg^{-1}, respectively (Table 1).

In contrast, indomethacin decreased the level of PGE_2 in a dose-dependent manner, in inflammatory exudate, gastric mucosa and renal papillary tissue, with the same dose range, the ID50 being 0·23, 0·14 and 0·15 mg kg^{-1}, respectively (Table 1).

The same tendency was seen for 6-keto-$PGF_{1\alpha}$ and TXB_2. The concentration of prostanoid in inflammatory exudate, gastric mucosa and renal papillary tissue was $45{\cdot}7 \pm 7{\cdot}2$ ng/rat, 340 ± 24 ng (g tissue)$^{-1}$ and $3{\cdot}6 \pm 0{\cdot}2$ μg (g tissue)$^{-1}$ for 6-keto-$PGF_{1\alpha}$, and $14{\cdot}2 \pm 1{\cdot}8$ ng/rat, $31{\cdot}3 \pm 9{\cdot}2$ ng (g tissue)$^{-1}$ and $0{\cdot}11 \pm 0{\cdot}01$ μg (g tissue)$^{-1}$ for TXB_2. The ID50 values of NS-398 and indomethacin for each prostanoid are summarized in Table 1.

The total number of cells in the inflammatory exudate was $7{\cdot}2 \pm 1 \times 10^6$, mainly neutrophil and basophil-like cells (about 50% and 40% of total cells, respectively), obtained 3 h after carrageenan injection. The number of neutrophils significantly decreased on treatment with 10 mg kg^{-1} NS-398 or indomethacin, whereas the other cell types (basophil-like cells, acidophils and lymphocytes) were not significantly affected.

The effects of NS-398 and indomethacin on PGE_2 production in inflamed and non-inflamed tissue are shown in Table 2. Inflamed tissue exhibited about 3–7 times the PGE_2 production than did non-inflamed tissue. NS-398, 30 mg kg^{-1}, inhibited the excess PGE_2 production in inflamed tissue to the level seen in non-inflamed tissue. Nevertheless, NS-398 had no effect on the PGE_2 production in non-inflamed tissue.

In contrast, indomethacin, 3 mg kg^{-1}, markedly inhibited PGE_2 production in inflamed and non-inflamed tissues, to a similar degree.

Discussion

We have reported that NS-398 given in a single administration of 1000 mg kg^{-1} caused very few gastric lesions, yet the anti-

Table 1. Effects of NS-398 and indomethacin on prostanoid production in carrageenan-air-pouch rats.

	ID50 (mg kg^{-1})			Relative potency	
	Exudate (A)	Gastric mucosa (B)	Renal papillary tissue (C)	B/A	C/A
PGE_2					
NS-398	0·18	62·2	261·7	346	1454
Indomethacin	0·23	0·14	0·15	0·61	0·65
6-keto-$PGF_{1\alpha}$					
NS-398	0·06	>1000	166·5	>1000	2775
Indomethacin	3·35	6·22	1·24	1·86	0·37
TXB_2					
NS-398	0·33	>1000	>1000	>1000	>1000
Indomethacin	0·20	1·34	7·92	6·70	39·6

ID50 values are defined as the dose inhibiting the control value by 50%. Relative potency was calculated from the ID50 values.

Table 2. Effects of NS-398 and indomethacin on PGE_2 production in inflamed and non-inflamed tissue in rats.

	PGE_2 production (%)	
	Non-inflamed	Inflamed
Control	100 ± 4·2	296 ± 48·2**
NS398	102 ± 3·6	107 ± 4·2##
Control	100 ± 17·7	683 ± 124**
Indomethacin	0·5 ± 2·6**	52·8 ± 12·0##

Drug, NS-398 at 30 mg kg^{-1} and indomethacin at 3 mg kg^{-1}, were administered orally 3 h before the assay. **Significantly different from the control of non-inflamed tissue ($P < 0.01$), determined by Dunnett's test. ## Significantly different from the control of inflamed tissue ($P < 0.01$), determined by Dunnett's test.

inflammatory, analgesic and antipyretic effects were as potent as those of indomethacin, diclofenac and loxoprofen (Futaki et al 1993). NS-398 also produced much weaker gastrointestinal and renal lesions, when given orally for 3 months (data not shown). Such a separation of side-effects from therapeutic effects was not seen with other NSAIDs. Some NSAIDs with a lesser ulcerogenicity have been developed as prodrugs (Misaka et al 1981; Kobayashi et al 1984). NS-398 functions directly, and not as a prodrug.

The rat carrageenan-air-pouch inflammation model has been extensively used to analyse inflammatory mediators. With this model, the PGE_2 content in inflammatory exudate increased, and was decreased by NSAIDs and anti-inflammatory steroids, in a dose-dependent manner (Sato et al 1980). Decrease in the level of PGE_2 in the exudate closely correlated with the suppression of vascular permeability, as a result of the inhibition of PGE_2 biosynthesis by the tissue. Thus, the decrease in the level of prostanoid seems to reflect the inhibition of prostanoid production in the tissue.

In carrageenan-air-pouch inflammation, NS-398 inhibited prostanoid production in the inflammatory exudate, to the same extent as seen with indomethacin, a finding which explains the anti-inflammatory effects of NS-398 in-vivo. On the other hand, in the gastric mucosa and the renal papillary tissue of the same rat, the inhibitory activity on prostanoid production by NS-398 was much weaker than that seen in the inflammatory exudate (Table 1). Thus, the inhibitory effect of NS-398 is more specific for inflamed tissue than for gastric mucosa and kidney. Moreover, in the gastric mucosa, PGI_2, which has gastro-protective effects like other prostaglandins, is the major cyclo-oxygenase product. NS-398 did not affect the concentration of 6-keto-$PGF_{1\alpha}$ in gastric mucosa, hence the low ulcerogenicity and renal side-effects of NS-398 can be explained.

Effects of indomethacin on prostanoid production differed from findings with NS-398. Indomethacin inhibited PGE_2 production in inflammatory exudate, gastric mucosa and renal papillary tissue, to the same degree. The ratios between the ID50 for gastric mucosa and renal papillary tissue and that for inflammatory exudate were 0·61 and 0·65, respectively, thereby reflecting the marked toxicity of indomethacin. The same was noted for 6-keto-$PGF_{1\alpha}$ and for TXB_2 (Table 1).

Nimesulide and pranoprofen have also been reported to have a selective inhibition on PGE_2 production in inflamed tissue, but the ratio between the inhibitory activity for the inflammatory exudate and for the gastric mucosa was 12·4 for nimesulide (Tofanetti et al 1989), and 25 for pranoprofen (Imayoshi et al 1984). NS-398 has a much greater specificity in inhibiting prostanoid synthesis.

To clarify mechanisms involved in the specific inhibitory activity of NS-398, we compared the inhibitory effect of PGE_2 production between inflamed and non-inflamed tissue from the same area of rat dorsum. Inflamed tissue induced by carrageenan showed about a three to seven-fold PGE_2 production than did non-inflamed tissue. This observation is in agreement with reports that prostanoid production was enhanced (up-regulated) in the inflammatory stage. In inflamed tissue, NS-398, at 30 mg kg^{-1}, inhibited the excess PGE_2 production to the level seen in non-inflamed tissue, but not completely. Even with an overdose, NS-398 did not affect PGE_2 production in non-inflamed tissue. These findings suggest that NS-398 affects the excess (pro-inflammatory) prostaglandin production in the inflamed stage, but has little effect on the ordinary (cytoprotective) prostaglandin production. In contrast, indomethacin at the anti-inflammatory dose of 3 mg kg^{-1}, showed a distinct inhibition of PGE_2 production in both inflamed and non-inflamed tissues, and with no selectivity.

The mechanisms involved in the specificity of NS-398 for inflamed tissue are unclear. Several types of agonists induce increased prostaglandin production by synthesizing a new prostaglandin endoperoxide synthase enzyme, in cell culture systems (DeWitt 1991). The enzyme expression was up-regulated in inflammatory joint diseases and was down-regulated by anti-inflammatory glucocorticoids in-vivo (Sano et al 1992). This inducible cyclo-oxygenase differed from a constitutive cyclo-oxygenase. NS-398 has the potential to inhibit the new cyclo-oxygenase selectively without affecting a constitutive enzyme.

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